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Determination of Multi-Drug Resistant and Extended Beta-Lactamase Producing Bacteria at Bethezatha Advanced Laboratory, Addis Ababa, Ethiopia

Tenssay ZW* and Gebre A

Bethezatha Advanced Laboratory, Ethiopia

Abstract

Background: Antimicrobial resistant bacteria are global health problems. Bacteria have several mechanisms to resist drug effects. Extended Spectrum Beta Lactamase (ESBLs) represents an impressive example of the ability of gram-negative bacteria to develop new antibiotic-resistance mechanisms in the face of the introduction of new antimicrobial agents. Assessing ESBL producing Enterobacteriaceae in the local scenario is necessary to understand the epidemiology and the disease burden as well as to design and implement hospital infection control strategies to prevent the further occurrence and spread of such bacteria.

Materials and Method: A retrospective study of different clinical specimens (urine, blood culture, pus and discharges from different body sites) received by Bethzatha Advanced Laboratory for bacteriological culture and antimicrobial susceptibility test from different wards of Bethzatha Hospital and other Health Institutions in Addis Ababa. These were cultured for isolation and identification of bacterial pathogens using standard bacteriological culture media. MicroScan panels (Beckman Coulter, Brea, CA, USA) method was used for antibiotic susceptibility test and for extended spectrum bata-lactamase (ESBL) detection.

Results: A total of 2112 clinical specimens were cultured at Bethzatha advanced Laboratory from May 2021 to July 2023. Most frequent isolate, 126 (58.6%) was *E. coli*. Of the total 216 gram-negative bacilli, 109 (51%) was found to be ESBL producers. Out of these, *E. coli* 68 (53.9%) were ESBLs producer followed by 21/41 (51%) *Kllebsiella pneuomoniae*, 9/19 (47.4%) *P. mirabilis* and 5/10 (50%) Enterobacter cloacae. Among ESBLs producing *E. coli*, 15/68 (22%) was resistant to six different antimicrobial drugs and one strain was resistant to 12 or more antimicrobials tested. Similarly, 4/21 (19%) and 2/5 (40%) *K. pneumoniae* and *K. oxytoca* were respectively resistant to six or more antimicrobial agents.

Conclusion: In the present study multiply resistant and ESBL producing Gram negative bacilli were frequently isolated from different clinical specimens. Therefore, there must be periodic surveillance of antimicrobial resistance in hospitals and other health settings. Appropriate prescription of antimicrobial agents and avoiding misuse of antimicrobials is also important to minimize the spread of ESBL producing multidrug resistant bacterial strains.

Keywords: MicroScan Pannel, Enterobacteriaceae; Extended Spectrum Beta Lactamase (ESBL); Antimicrobial Resistance

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Correspondence:
Zeleke W Tenssay, Bethezatha Hospital, Addis Ababa, Ethiopia
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Introduction

Antimicrobial resistant bacteria are global health problems causing 700,000 deaths annually and it has been predicted that if appropriate control and prevention measures are not taken Antimicrobial Resistance (AMR) would become one of the main reasons of death among hospitalized and non-hospitalized patients in developing and developed countries [1]. Proper antibiotic usage and administration are essential for treatment of bacterial infections. Thus, inappropriate prescription and misuse of antibiotics could contribute to emergence of AMR pathogenic bacteria, restriction of therapeutic options, increase of hospitalization time and high treatment costs and finally a greater death rate [2].

Bacteria have several mechanisms to resist drug effects. Some change the target point so that drugs cannot get the target, others produce enzymes to make the drug ineffective, yet others change

their metabolic pathways etc. Although the discovery of penicillin a class of beta-lactam antibiotic made a great triumph over infectious diseases caused by many bacteria, it was not very long that penicillin resistant staphylococci and other target organisms emerged with beta lactamase enzyme to hydrolyze the drugs and resist the antibiotics. The age of penicillin faced the rapid emergence of resistance in *Staphylococcus aureus* due to a plasmid-encoded penicillinase. This β-lactamase quickly spread to most clinical isolates of *S. aureus* as well as other species of staphylococci [3]. Researchers dealing with microbial drug resistance mechanisms continuously tracking the changing antimicrobial resistance mechanism in such bacteria have made enormous efforts to meet the challenges. The introduction of the third-generation cephalosporin into clinical practice in the early 1980s was such a successful effort as a major breakthrough in the fight against -lactamase-mediated bacterial resistance to antibiotics [4]. The cephalosporin had been developed in response to the increased prevalence of beta-lactamases in certain organisms such as *Escherichia coli* and *Klebsiella pneumoniae* and the spread of these beta-lactamases into new hosts. Not only were the third generation cephalosporins effective against most beta-lactamase-producing organisms but they had the major advantage of lessened nephrotoxic effects compared to aminoglycosides and polymyxins.

Nevertheless, the successful treatment of certain bacterial infections by cephalosporin, have recently been challenged because of bacteria that produce Extended Spectrum Beta-Lactamases (ESBLs). Currently used definition for ESBL is β-lactamase that is able to render the bacterium resistant to the penicillin, first, second, and third-generation cephalosporins and aztreonam, but not cephamycin's or carbapenems, by hydrolysis that could be inhibited by β-lactamase inhibitors of these antibiotics [5]. ESBLs represent an impressive example of the ability of gram-negative bacteria to develop new antibiotic-resistance mechanisms in the face of the introduction of new antimicrobial agents. Extended-Spectrum β-Lactamases (ESBLs) are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid. They represent the first example in which β-lactamase–mediated resistance to β-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes. ESBLs have become widespread throughout the world and are now found in a significant percentage of *Escherichia coli* and *Klebsiella pneumoniae* strains in certain countries. They have also been found in other Enterobacteriaceae strains and *Pseudomonas aeruginosa*. Strains expressing these β-lactamases will present a host of therapeutic challenges as we head into the 21st century [5].

Assessing ESBL producing Enterobacteriaceae in the local scenario is necessary to understand the epidemiology and the disease burden as well as to design and implement hospital infection control strategies to prevent the further occurrence and spread of such bacteria. Furthermore, the prevalence of ESBL in Enterobacteriaceae could be variable according to the species, hospital allocation, sites of infection, as well as nosocomial or community acquisition [6]. Despite the need to determine the occurrence of ESBL producing bacteria, reports on ESBL producing multidrug resistance Enterobacteriaceae is not common in low- and middle-income countries like Ethiopia. Moreover, clinical bacteriology laboratories performing ESBL tests are very scarce in Ethiopia because of lack of laboratory techniques. Therefore, in Ethiopia, there are very few studies on ESBLs producing bacterial isolates [7,8]. Among the frequently isolated bacterial pathogens from clinical samples are Enterobacteriaceae such as *E. coli*, *Klebsiella* spp., *Enterobacter* sp. and *Proteus* spp. [9-12]. Some of these enteric bacteria have been found to produce extended spectrum Beta lactamase causing pediatric septicemia, urinary tract infection and surgical site infections [7]. Moreover, most of these bacterial pathogens are multidrug resistant and it is important to detect the ESBL producers. Therefore, the objective of this work is to determine ESBL producing gram negative bacteria from different clinical specimens received for microbiological diagnosis by Bethzatha Advanced Laboratory.

Materials and Methods

Specimen and bacteriological culture

Different clinical specimens (urine, blood culture, pus and discharges from different body sites) sent to Bethzatha Advanced Laboratory for bacteriological culture and antimicrobial susceptibility test from different wards of Bethzatha Hospital and other Health Institutions in Addis Ababa. These were cultured for isolation and identification of bacterial pathogens and antimicrobial susceptibility testing. The cultures were done on conventional culture media such as MacConkey, Blood agar, Nutrient Agar, Mannitol salt agar, Chocolate agar and Salmonella-Shigella agars depending on the types of the specimen by Cheesbrough [13].

Bacterial identification and antimicrobial susceptibility test

Bacterial identification, antimicrobial susceptibility test and detection of ESBLS producers were done using Semi automated method known as MicroScan Panel Identification methods (Beckman Coulter, Brea, CA, USA). According to this method identification of gram-negative organisms is done by inoculating dried overnight Negative COMBO and identification of gram-positive organisms by inoculating dried overnight positive COMBO panels. MicroScan dried overnight COMBO Panel is a Panel containing both dried biochemical reagents and antimicrobial agents.

Detection of ESBL

There are different CLSI recommended methods for detection of ESBL producers such as disk diffusion, Minimum Inhibitory Concentration (MIC) break point, and commercially available Methods such as MicroScan panels which contain combinations of ceftazidime or cefotaxime plus-lactamase inhibitors have received Food and Drug Administration approval and in studies of large numbers of ESBL-producing isolates [14].

MicroScan panels (Beckman Coulter, Brea, CA, USA) comprise dehydrated panels for microdilution antibiotic susceptibility and for ESBL Detection. MicroScan COMBO Panels, can test susceptibility up to 28 different antimicrobials by the minimum inhibitory concentration methods, with break point referenced to CLSI (Clinical and Laboratory Standard Institute) guide line.

According to MicroScan Panel identification system 3 to 4 pure bacterial colonies were picked from 18 h to 24 h aerobic culture by means of a wand designed for holding bacterial material from primary isolation media mentioned above and inoculated into 30 ml of prompt inoculation water (Beckman Coulter, Brea, CA, USA). Then the bacterial suspension was transferred into Seed Tray Inoculator D sets (Beckman Coulter, Brea, CA, USA). The COMBO panel wells are inoculated from bacterial suspension in the Seed Tray using a device known as MicroScan Renok (Beckman Coulter, Brea, CA, USA) which delivers 115 µL of broth suspension to each well. According

to manufacturer's instruction (Beckman Coulter, Brea, CA, USA) three drops of mineral oil was added to the wells containing glucose, urea, lysine, H_2S, arginine, ornithine, for gram negative COMBO panels; and for gram positive COMBO Panels only arginine and urea containing wells were overlaid with the mineral oil. Some reagents recommended by the manufacturer were added to the panels after incubating for 18 h to 24 h at 35°C aerobically.

The panels were read by MicroScan AutoScan 4 automated reader (Beckman Coulter, Brea, CA, USA). The MicroScan automated reader gives the identification for each bacterial biotypes with probability scores. Results with high probability scores (>85%) were considered reliable while results with probability scores (<85%) "unconfirmed". If the biochemical profile did not much any identification in Program's software database, the result generated was "very rare bio type". Compared to the manual biochemical identification conventionally used in traditional microbiology laboratory of low-resource settings, diverse bacterial biotypes were generated by the automated system. At the same time antimicrobial susceptibility and ESBL is read by the MicroScan AutoScan 4.

Results

A retrospective study of a total of 2,112 clinical specimens sent for culture and antimicrobial susceptibility tests to Bethzatha Advanced Laboratory from May 2021 to July 2023 were conducted. The most frequent specimens were, urine 491 (23%), blood culture 542 (28%), pus and discharges from different body sites 300 (14%), and body fluid including cerebrospinal fluid 411 (19%).

Out of the total 2,112 clinical specimens, 592 (28%) yielded 235 (39.7%) gram positive and 339 (57%) gram negative bacterial pathogens. Of the 339 gram-negative bacteria, 215 were *E. coli*, *Klebsiella pneumonia*, *K. oxitoca*, *P. mirabilis* and *Enterobacter cloacae*. These gram-negative bacteria were analyzed for Extended Spectrum Beta Lactamase (ESBL) production. Table 1 shows ESBL producing and non-producing Enterobacteriaceae. The most frequent 126 (58.6%) gram negative bacterial isolates were *E. coli*. Out of this *E. coli* 68 (53.9%) were found to be ESBLs producers, followed by 21/41 (51%) *Kllebsiella pneuomonia* and 9/19 (47.4%) *P. mirabils* (Table 1).

The clinical sources of the ESBLs producing Enterobacteriaceae is depicted on Table 2. Most 53 (78%) of the ESBL producing *E. coli* were isolated from urine. Similarly, the majority 7/20 (35%) *K. pneumoniae*, 4/5 (80%) *K. oxytoca*, and 8/9 (89%) *P. mirabilis* were isolated from urine (Table 2). Most of these bacterial isolates were multidrug resistant.

Table 3 shows antimicrobial resistance patterns of most common gram-negative bacterial isolate in the present study. In the present study, *E. coli* was most frequent isolate 126 (57%) of the total gram-negative bacteria from different clinical specimens. *E. coli* was multiply resistant to most antimicrobial drugs tested and 57 (45%) were resistant to ampicillin sulbactam, 28 (22%) to amoxicillin clavulic acid. Similarly, *K. pneumoniae* 41/220 (18.6%) was next to *E. coli* in frequency. *K. pneumoniae* 33/41 (80%), 31/41 (75%), 25/41 (61%) were respectively resistant to cefepime, cefuroxime, and ciprofloxacin (Table 3).

The level of resistance of these isolates is shown on Table 4. Out of 126 *E. coli* isolates, 18 (14%) were resistant to 12 or more antibiotics tested; similarly, 6/41 (15%), 5/19 (26%) and 5/16 (31%) *K. Pneumoniae*, *K. oxytoca*, and *P. mirabillis* were respectively resistant to 12 or more drugs (Table 4). Resistance level of ESBLs producer is depicted on Table 5. The ESBL producers are multiply resistant to antimicrobial drugs tested. All the ESBLs producing isolates have shown resistance to at least one drug. Six (5.6%), 7 (6.5%), 23 (21.3%) have shown resistance to three, four and six different antimicrobial drugs respectively, and 4 (3.7%) isolates have resistance to 12 or more drugs (Table 5). Among ESBLs producing *E. coli* 15/68 (22%) were resistant to six different antimicrobial drugs and one strain was resistant to 12 or more antimicrobials tested. Similarly, 4/21 (19%) and 2/5 (40%) *K. pneumoniae* and *K. oxytoca* were respectively resistant to six antimicrobial agents.

Discussion

In the present study, out of 216 gram-negative bacterial species 109 (50.5%) were detected to be ESBL producers. *E. coli* and *Klebsiella pneumonia* were found to be the dominant Extended Spectrum Beta Lactamase (ESBL) producing gram negative bacilli. Other ESBL

Table 1: Extended beta lactamase producers *vs.* non-producer Enterobacteriaceae isolates May 2022 to July 2023.

Isolates	ESBL producers' number (%)	ESBL non producers' number (%)	Total number (%)
E. coli	68 (53.9)	58 (46.0)	126 (58.6)
K. pneumoniae	21 (51)	20 (48.7)	41 (18.6)
K. oxytoca	5 (31)	11 (68.8)	16 (7.4)
P. mirabilis	9 (47.4)	10 (52.6)	19 (8.8)
E. cloacae	5 (50)	5 (50)	10 (4.7)
S. marscens	1 (25)	3 (75)	4 (1.8)
Total	109 (50.5)	107 (49.5)	216 (100)

Table 2: Clinical sources of ESBL producing isolates.

Specimen	*E. coli* 126/215	*K. pneumoniae* 41/215	*K. oxytoca* 16/215	*E. cloacae* 10/215	*P. mirabilis* 19/215	*S. marscens* 4/215
Urine	53 (78)	7 (35)	4 (80)	4 (80)	8 (88.9)	0 (25)
Blood	4 (5.9)	6 (30)	1 (20)	0 (0)	0 (0)	1 (100)
Pus	9 (13.2)	3 (15)	0	1 (20)	1 (11.1)	0 (0)
Others	2 (2.9)	4 (20)	0	0	0 (0)	0 (0)
Total	68 (53.9)	20 (48.8)	5 (31)	5 (50)	9 (47.4)	1 (25)

Table 3: Distribution of antibiotics resistance among Enterobacteriaceae isolates.

Isolates	Antimicrobials																	
	ak	amo/c	amp/su	azt	cefp	tob	cfz	cefu	cip	col	gn	ertp	imp	lev	mer	pip/t	tig	cot
E. coli 126/220	7 (5.6)	28 (22)	57 (45)	12 (10)	24 (19)	12 (10)	11 (9)	22 (17)	20 (16)	8 (6)	10 (8)	12 (10)	9 (7)	21 (17)	11 (9)	12 (10)	2 (2)	24 (19)
K. pneumoniae 41/220	7 (17)	17 (42)	27 (66)	18 (44)	33 (80)	23 (56)	15 (37)	31 (75)	25 (61)	14 (34)	25 (61)	16 (39)	13 (31)	14 (34)	14 (34)	12 (29)	3 (7)	31 (75)
K. oxytoca 16/220	1 (6.3)	7 (44)	9 (56)	9 (56)	12 (75)	7 (44)	6 (38)	11 (69)	4 (25)	3 (18)	6 (38)	8 (50)	6 (38)	4 (25)	6 (38)	5 (31)	0	11 (69)
E. cloacae 10/220	1 (10)	8 (80)	6 (60)	3 (30)	8 (80)	0	4 (40)	8 (80)	5 (50)	2 (20)	3 (30)	3 (30)	(10)	2 (20)	1 (10)	1 (10)		4 (40)
E. aerogenes 2/220	0	0	1 (50)	1 (50)	1 (50)	0	0	2 (100)	0	1 (50)	0	0	0	0	0	0	0	0
S. marscens 4/220	0	3 (75)	1 (25)	1 (25)	2 (50)	0	1 (25)	2 (50)	0	3 (75)	0	1 (25)	0	0	0	0	0	0
P. mirabilis 19/220	4 (21)	5 (26)	5 (26)	11 (58)	17 (89)	12 (63)	8 (42)	14 (73)	16 (84)	19 (100)	12 (63)	8 (42)	10 (53)	10 (53)	2 (11)	3 (16)	0	14 (74)
P. vulgaris 3/220	1 (33)	0	1 (33)	2 (67)	3 (100)	1 (33)	2 (67)	2 (67)	1 (33)	3 (100)	1 (33)	1 (33)	0	1 (33)	0	0	0	1 (33)

Ak: Amikacin; amo/cl: Amoxicillin clavulnate; am/sul: ampicillin sulbactam; azt: azeromycin; cefp: cefpime; tob: tobramycin; cez: ceftazadime; cfu: cefuroxime; cip: ciprofloxacine; col: colesine; gn: gentamicine; ertp: ertapenem; imp: impipenem; lev: levofloxacine; mrp: meropenem; pip/t: piperacillintazobactam; tig: tigycycline; cot: trimethoprim sulfamethoxazole

Table 4: Level of antibiotics resistance among Enterobacteriaceae tested.

Solates	NO	R_0	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	$R_{12\geq}$
E. coli	126	3	3	6	10	5	8	17	9	7	2	2	1	18
K. pneumoniae	41	4	3	0	0	1	4	7	2	2	1	1	2	6
K. oxytoca	16	1	1	2	1	0	1	1	2	0	1	0	1	5
Enterobacter cloacae	10	0	0	1	1	0	1	1	1	2	1	0	1	0
P. mirabilis	19	0	0	1	0	1	0	1	3	4	2	2	0	5
Serratia marcescens	4	0	1	0	0	1	1	0	0	0	0	0	0	0
Total	215	8 (3.7)	8 (3.7)	10 (4.6)	12 (6.9)	8 (3.7)	15 (6.9)	27 (12.6)	17 (7.9)	15 (6.9)	7 (3)	5 (2)	5 (2)	34 (15.8)

Level of resistance to antimicrobials, R0, R1, ……. R12

Table 5: Level of antibiotics resistance among ESBL producing Enterobacteriaceae.

Solates	NO	R_0	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	$R_{12\geq}$
E. coli	68	0	1	2	4	2	4	15	9	5	3	1	1	1
K. pneumoniae	21	0	0	0	1	2	5	4	2	2	2	1	1	1
K. oxytoca	5	0	0	0	0	1	0	2	1	1	0	0	0	1
Enterobacter cloacae	5	0	0	0	1	0	0	0	1		1	0	1	0
P. mirabilis	9	0	0	0	0	1	0	2	1	1	0	1	0	1
Serratia marcescens	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Total	108	0	1 (0.9)	2 (1.9)	6 (5.6)	7 (6.5)	9 (8)	23 (21)	14 (13)	9 (8)	6 (5.6)	3 (2)	3 (2)	4 (3.7)

Level of resistance to antimicrobials, R0, R1, …….. R12

producing Enterobacteriaceae included *K. oxytoca*, *Enterobacter cloacae*, *P. mirabilis* and *Seratia marscens*. These bacteria were isolated frequently from clinical samples: Urine, pus, blood culture and others specimens such as body fluid. In the present study, ESBL producing *E. coli* 53/68 (78%) was isolated from urine samples. *Klebsiella pneumonia* 7/21 (33%) was the next frequent ESBL producing isolate from urine. Out of 16 *K. oxytoca*, 5/16 (31%) were ESBL producers and isolated 4/5 (80%) of urine samples. Meeta Sharma et al. [15] studied prevalence and antibiogram of extended spectrum beta-lactamase producing gram negative bacilli from rural India and found that 52.5% of the isolates were ESBL producers which is comparable to the present finding, but the major source of the ESBL producers in that study was isolated from respiratory tract. *Klebsiella* species were the most dominant (67.4%) ESBL producing bacteria in the same study. The present finding of highest per cent of ESBL producing *E. coli* from urine sample fits to the previous reports by Abayineh et al. [16] from Jimma University, south west Ethiopia and from Northern Thailand [17] and Cambodia [18]. Our finding of 53% (68/126) *E. coli* and 51% (21/41) ESBL producing *K. pneumoniae* is higher than 42% and 30% reported from Northern Thailand [17]; 13.51% (60/444); 16.55% (24/145) from Nepal [19]; and 42.7% and 33.7% reported for the same organisms from Cambodia [18]. On the other hand, the sample size of the previous workers was relatively larger than the present samples. However, 76% (13/17) ESBL producing *E. coli* reported from Jimma University south west Ethiopia is higher than the present finding. In the present study all of the ESBL producing Enterobacteriaceae isolates were multidrug resistant to two or more drugs tested. The current finding is in agreement with previous works from Ethiopia [16] and elsewhere in the world [15,17-19]. In the present study 33/41(80%), 31/41 (75%) and 27/41 (66%) *K. pneumoniae* isolates were multidrug resistant to cefepime, cefuroxime and cotrimoxazole and ampicillin- sulbactam respectively. Ameshe et al. [7] studied antimicrobial resistance patterns and production of extended beta lactamase in *Klebsiella* species isolated from among UTI suspected patients in Bahirdar city, north-west Ethiopia detected a similar pattern of multidrug resistance to the present finding. Various

workers from Ethiopia and from other parts of the world reported increasing spread of antimicrobial resistance problems stemming from ESBL production among common Gram-negative bacteria [7,15,20].

Limitations

In the present study the sample size was relatively small, and demographic data are not included, however the results indicated that there is a high rate of ESBL producing and multiple antimicrobial resistant gram-negative bacterial strains in the settings of studied hospitals and other health institutions.

Conclusion

Extended-Spectrum β-Lactamases (ESBLs) are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid. They represent the first example in which β-lactamase–mediated resistance to β-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes. ESBLs have become widespread throughout the world and are now found in a significant percentage of *Escherichia coli* and *Klebsiella pneumoniae* strains in certain countries. They have also been found in other *Enterobacteriaceae strains*, *Enterobacter cloacae*, *Proteus mirabilis*, *Seratia marscens* and *Pseudomonas aeruginosa*. The present finding confirms the previous reports from Ethiopia that ESBL producing multiple antimicrobial resistant Enterobacteriaceae are rapidly spreading in Ethiopia as in other developing counties. The increase in ESBL producing and multiple antimicrobial resistant gram-negative bacteria from clinical specimens is a threat to infection control and limit treatment options in low-income countries like Ethiopia. Therefore, there must be periodic surveillance of antimicrobial resistance in hospitals and other health settings because most of these bacteria can be hospital as well as community acquired. Furthermore, microbiology laboratories must be strengthened with necessary facilities and skilled professionals in order to make correct and regular diagnosis and maintain sustainable reporting system. Appropriate prescription of antimicrobial agents and avoiding misuse of antimicrobials is also important to minimize the spread of ESBL producing multidrug resistant bacterial strains.

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